Comparative Research on EPS Extraction from Mechanical Dewatered Sludge with Different Methods

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Abstract: *In order to find a suitable extracellular polymer substance (EPS) extraction method for mechanical dewatered sludge, four different methods including EDTA extraction, alkali extraction, acid extraction, ultrasonic extraction method have been used in extracting EPS from belt filter dewatered sludge. The contents of polysaccharide and proteins extracted from the dewatered sludge by different extraction methods are also analyzed. The results indicated that EDTA method and alkali extraction method are more suitable for dewatered sludge with more EPS content and less cell damage, while sulfuric acid extraction and ultrasonic extraction were poorer with obvious cell lysis shown by higher DNA content in extracted EPS. Contents of proteins and polysaccharide in EPS extracted from mechanical dewatered sludge, is at the contents between that in EPS extracted from activated sludge and anaerobic digestion sludge.*

Keywords: *Mechanical dewatered sludge, Extracellular polymeric substances, Extraction methods.*

Introduction

Mechanical dewatered sludge is an inevitable product of wastewater treatment. The statistics from relevant department showed that output of mechanical dewatered sludge with water content of 80% was up to 33 million tons in 2014 and continued to grow by 13% a year [13]. High moisture content is the bottleneck of sludge disposal. And most water in mechanical dewatered sludge exists in Extracellular Polymeric Substance (EPS) [12, 23]. Since further eliminate the moisture in dewatered sludge is important to sludge treatment and disposal, it is really necessary to know the characteristics of EPS in sludge.

EPS is the general term of a complex high-molecular-weight mixture of microorganism polymers, it is the products of cellular lysis, hydrolysis of macromolecules and wastewater adsorption, with the characteristic of negative charge and gel-like matrix with high water contents [20]. EPS can be sequestrated a long time in microorganisms, form the stable microbial colonies and the molecular weight of EPS is from thousands to millions [14]. As the main components in EPS, proteins and polysaccharide have accounted for about 70 to 80% of its total amount [6, 17]. About 50 to 70 percent organic matter in activated sludge is EPS, which indicate that EPS is the major component of sludge flocs [14]. EPS largely affects the sludge floc structure, sludge sedimentation and dehydration performance. By analyzing the influences of protein and polysaccharide distributions from different EPS extraction methods, it can provide the theoretical basis for the in-depth study of sludge moisture distribution relationship and the related research of the sludge nature.

The quantity and quality of EPS extraction is not only related to sludge types, but also depend on EPS extraction methods used [3, 9]. Till now, no standard EPS extraction has been established in academia because different methods have different efficiency [2, 8]. High extraction efficiency, low cell lysis maximum extraction, low chemical pollution and short extraction cycle should be taken into consideration when evaluate different EPS extraction methods [8, 15]. The three substances, including protein, polysaccharide and nucleic acid, determine the amounts of extracted EPS [19], and the ratio of nucleic acid and polysaccharide content has been regarded as an index for estimate the cell damaged degree in the process of EPS extraction [1, 18].

Currently, most EPS extraction research focus on the activated sludge and the anaerobic digestion sludge, while this study take sample from the belt filter dewatered sludge from a wastewater treatment plant. Thus we can compare the EPS extraction results of the dewatered sludge with the activated sludge. So this research can provide references for the dewatered sludge EPS extraction by analyzing the potential impact of sludge treatment process for the sludge and offering data reference for the sludge treatment and further applications.

Material and methods

Source of sludge

The sludge sample was from belt filter dewatered sludge, which collected from a wastewater treatment plant in Dalian, China. The experiments with the sludge sample were finished within three weeks. The water content of the sludge sample is 80.06%, and the water content was adjusted to 95% before experiment, two sets of parallel samples were adopted in each method.

Major chemical reagents

EDTA-2Na (AR), NaOH (AR), H_2SO_4 (AR), Anhydrous glucose (GR), Anthranone (AR), Coomassie Brilliant Blue (CBB) (AR), Absolute ethyl alcohol (AR), Bovine serum albumin (BSA) (Sigma), Calf thymus (Sigma).

Experimental apparatus

Visible light spectrophotometer; WF2100 (UNICO); UV VIS spectrophotometer; WFZUV-2000; constant temperature bath oscillator; thermostat water bath; ultrasonic cell crusher; high speed centrifuge.

Methods

EDTA method

The sludge sample (20 ml) was centrifuged by high speed centrifuge (3000 r/min) for 5 min, and removed the supernatant; 20 ml 0.9% of normal saline was added to wash the sediment according to the above method; then add 20 ml normal saline to the sediment which on the constant temperature bath oscillator, the glass homogenizer was used for breaking the sludge particle after the sludge solution were shaken up. After the homogenate, 1 ml of the sludge solution was removed to 10 ml with a test tube for the extraction of EPS. In addition, 3 ml of the sludge solution was placed into an oven (105 °C) drying to constant weight with a evaporating dish, then calculated 1.00 ml sludge contained in the solution of dry sludge quality, and recording for data processing. Add 9 ml of 2% EDTA to the test tube. The samples were shaken for 20 min and then placed it in the refrigerator at 4 °C for 4 hours. At last, the sample was centrifuged at 4000 r/min for 5 min and the supernatant was ready for further test.

Alkali extraction method

The sludge sample pretreatment was the same as the EDTA method. Add 9 ml 1% NaOH to the test tube on the table concentrator and oscillated for 1 h. The sample was centrifuged at 4000 r/min for 5 min later and the supernatant for the following test.

Sulfuric acid extraction method

The sludge sample pretreatment was the same as the above methods. 9 ml 8% H_2SO_4 was added to the test tube. Subsequent processing was the same as the alkali extraction method. The process of EDTA, Alkali and Sulfuric acid methods for EPS extraction was shown in Fig. 1.

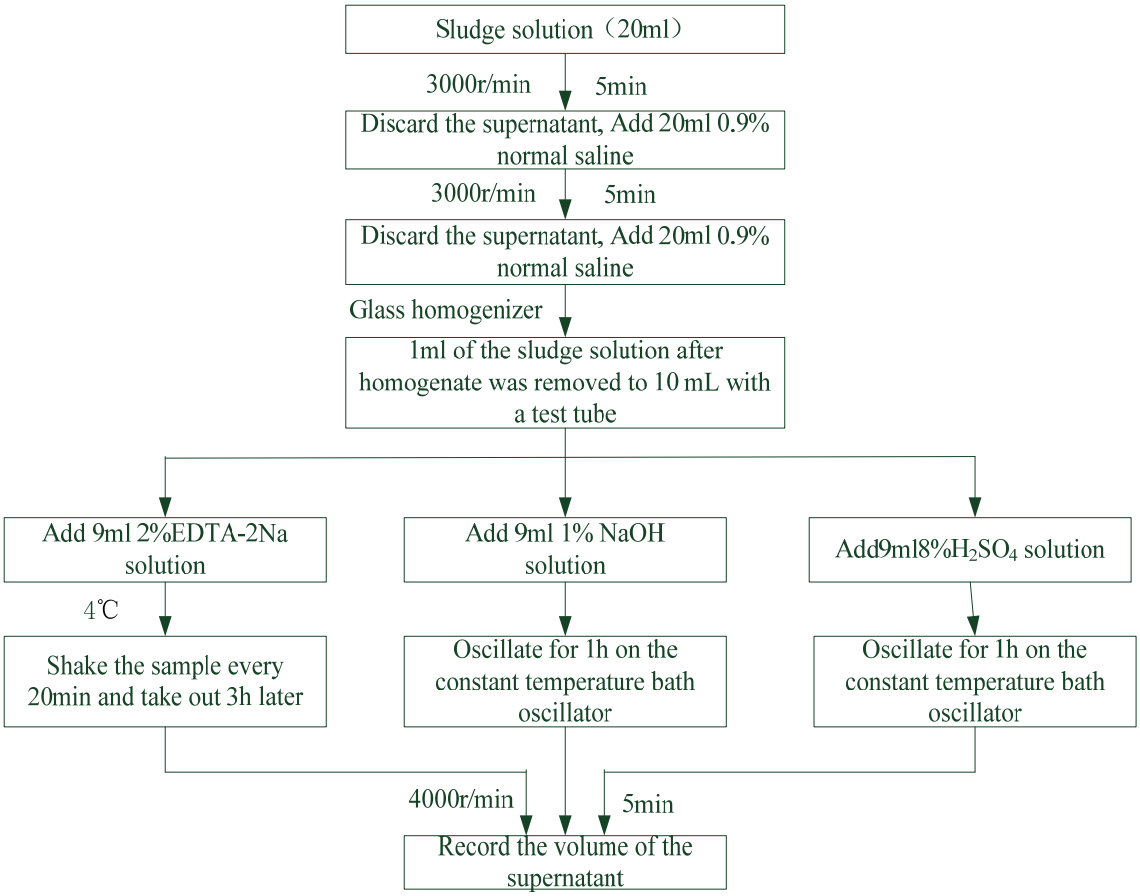

Fig. 1 The process of EDTA, Alkali and Sulfuric acid methods for EPS extraction

Ultrasonic extraction method

It will have the impact effect on the purity of EPS during ultrasonic process if using normal saline for the sludge washing, so the distilled water was used for sludge pretreatment, the samples were cleaned three times. Extraction using an ultrasonic cell crusher at the condition of 40 kHz, 100 W, then centrifuged at the speed of 6000 r/min for 20 min. The process of Ultrasonic methods for EPS extraction was shown as Fig. 2.

Chemical analysis of extracted EPS

As a general property production of microorganisms, EPS contains different organic macromolecules, such as proteins, polysaccharide and Nucleic acid [4] .The content of PS in EPS was measured by the anthrone sulfuric acid method [5] with glucose as the standard. PN content was determined by Bradford method with BSA (bovine serum albumin, Sigma) as the standard [21]. The Nucleic acid content was measured by the Ultraviolet spectrophotometer

method with calf thymus DNA (Beijing Solarbio Science & Technology Co., Ltd, China) as the standard. In Table 1 composition contents of EPS from four different extraction methods are presented.

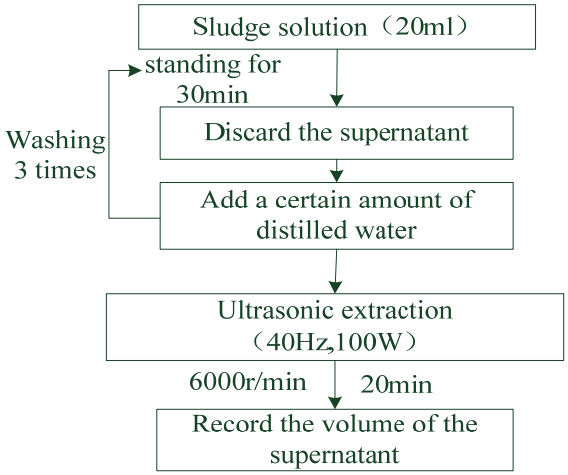

Fig. 2 The process of ultrasonic methods for EPS extraction

Table 1. Composition contents of EPS from four different extraction methods

Methods	Protein, mg/gvss	Polysaccharide, mg/gvss	Nucleic acid, mg/gvss
EDTA	2.80	66.57	22.51
NaOH	19.76	65.33	87.80
H_2SO_4	-	4.95	49.665
Ultrasonic	0.44	2.69	87.325

Results and discussion

The results of protein, polysaccharide and nucleic acid contents in EPS with different methods are shown in Fig. 3. The Fig. 3 shown that the protein concentration of four different extracted methods in descending order that, alkali extraction method > EDTA method > ultrasonic extraction method > sulfuric acid extraction method. The orders of polysaccharide concentration from different methods are, EDTA method > alkali extraction method > sulfuric acid extraction method > ultrasonic extraction method. The order of the nucleic acid concentration is that alkali extraction method > ultrasonic extraction method > sulfuric acid extraction method > EDTA method.

Compared the results of EDTA method with Liu et al. [11], the polysaccharide contents is 4 to 5 times as high as that of the latter, and the nucleic acid content is 1/8-1/9. Compared with Zheng et al. [22], protein contents is 2 times as high as that of the latter. Interestingly, extraction with EDTA method have given the highest EPS yields but the protein content is low, this might be explained by the fact that other complex substances were formed from protein during EDTA extraction, which decrease proportion of protein [10]. Such results can be explained that this experiment used the dewatered sludge which has more organic matters than anaerobic sludge in Liu's study. Under the anaerobic conditions, by facultative and obligate anaerobic bacterial degradation of organic matter occurs, the final product was carbon dioxide and methane gas. Therefore, some typical components in EPS, such as proteins and polysaccharides, might decompose in the process of anaerobic fermentation, and the sludge cellular was destroyed by

the anaerobic process, making the intracellular nucleic acid precipitate and increaing nucleic acid content in EPS.

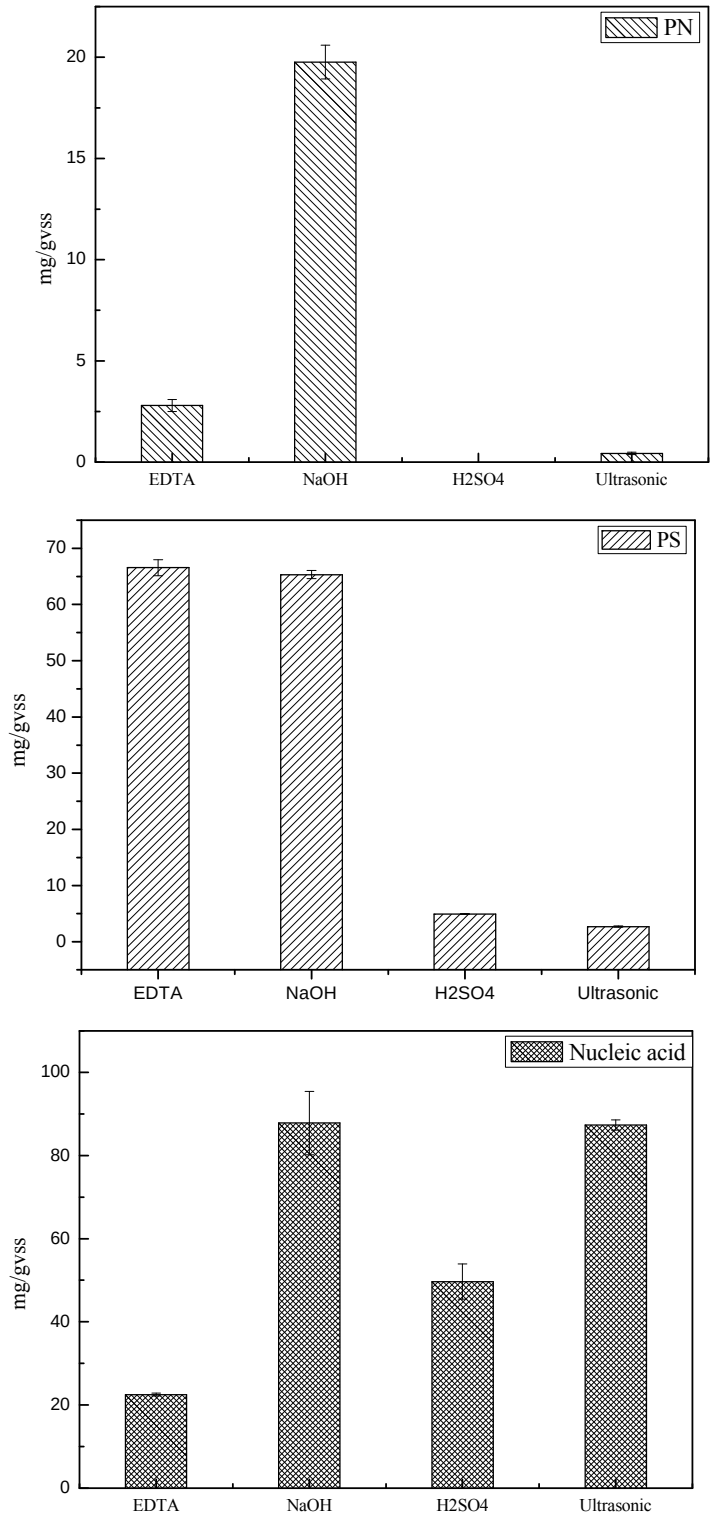

Fig. 3 The contents of proteins, polysaccharide and nucleic acid by four different extraction methods

The presence of NaOH may increase the pH value, which may lead to the dissociation of acidic groups and repulsion of negative charged extracellular polymeric substances [16], NaOH also increases water solubility of EPS which allowed more EPS to be extracted. Research in [9] declared that NaOH extraction method as the most efficient method. Compared with the results of Liu and Fang [10], polysaccharide content of Alkali extraction method is 2-3 times as high as that of the latter, and the nucleic acid content was less than the latter. Meanwhile, compared with the result of Zheng et al. [22], the protein content is much more than the latter. The reason is that colors of anthrone-sulfuric acid reagent for various hexoses, 6-deoxy-ribose are darker and colors for hexosamine, uronic acid are lighter. EPS contains many complex components such as hexose, pentose, as well as uronic acid. So values from anthrone-sulfuric acid method are lower. But polysaccharide concentration in the sample is high, which is due to excessive fragmentation cells, a large number of intracellular proteins, nucleic acids into the sample solution, tryptophan and other amino acids, which can react with anthrone – sulfuric acid reagent color reaction, might have interferences with results and make the measured values of partial polysaccharide 25% to 60% higher.

The results of sulfuric acid extraction method were compared with Liu, the protein and polysaccharide contents are, respectively, one-third and 1-2 times of the former study, the reason is some floc agents, such as polypropylene amide (PAM), can change the surface properties of the sludge and help release of colloidal proteins, polysaccharides and increase the content of proteins and polysaccharides in sludge filtrate, while nucleic acid content almost unchanged [7].

Compared with [6], the protein and polysaccharide contents of ultrasonic extraction results are half and one fifth of the former study, respectively, while the nucleic acid content is far exceed the results of [6]. This is by reason that the experimental conditions used in this study are 40 kHz, 100 W, while the latter is at the condition of 40 kHz, 50 W. High power conditions used may lead to the extremely high nucleic acid content value [17]. The content of protein and polysaccharide were less than the former study, reason maybe that the former study used activated sludge, which contains a large number of active microbial floc and extracellular polymeric than that of dewatered sludge.

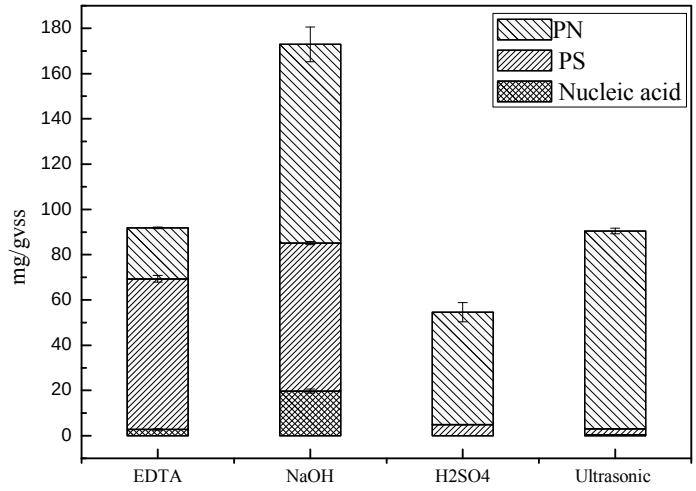

Fig. 4 The contents of EPS by four different extraction methods

Table 2. The ratio of nucleic acid and polysaccharide

EDTA	NaOH	H_2SO_4	Ultrasonic
0.203	0.806	6.026	19.478

Table 2 shows the ratio of nucleic acids and polysaccharides in dewatered sludge extracting solution, which contributes to quantitative measure of cell damage and the extraction efficiency of EPS. It can be evaluated of the four different extraction methods by Table 2 and Fig. 4 that, more EPS can be obtained from NaOH and EDTA extraction methods than that of sulfuric acid and ultrasonic extraction methods. However, high value of nucleic acid measured by alkali extraction method indicates more severely cells damaged than that of EDTA method. Protein content is not measured while using sulfuric acid extraction method, it can be explained that H_2SO_4 is serious interference in the determination of protein according to Liu and Fang [10]. Low ultrasonic extraction efficiency, high ratio of nucleic acid and polysaccharide were found in ultrasonic extraction method. So the EDTA method can be seen as the most appropriate method for EPS extraction of mechanical dewatered sludge. Since we can obtain EPS from mechanical dewatering sludge, then it is an interesting topic to relate EPS with moisture distribution for research of moisture elimination.

Conclusions

1) For belt mechanical dewatered sludge, the protein concentration of four different EPS extraction methods was: alkali extraction method > EDTA method > ultrasonic extraction method > sulfuric acid extraction method; Polysaccharide concentration order: EDTA method > alkali extraction method > sulfuric acid extraction method > ultrasonic extraction method; Nucleic acid concentration order: alkali extraction method > ultrasonic extraction > sulfuric acid extraction > EDTA method.

2) Sulfuric acid method have serious interference in the determination of protein content in EPS. The order of cell damage degree of four different EPS extraction methods was: ultrasonic extraction method > alkali extraction method > sulfuric acid extraction method > EDTA method.

3) The EDTA method is the most appropriate EPS extraction method for mechanical dewatering sludge.

Acknowledgements

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